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Computational Study of the Conformational Preferences of the (R)-8-Amino-pentacyclo(5.4.0.0^{2,6}.0^{3,10}.0^{5,9}) undecane-8-carboxylic Acid Monopeptide

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Abstract: α-Amino acids are important building blocks for the synthesis of a large number of bioactive compounds and pharmaceutical drugs. However, a literature survey revealed that no theoretical conformational study of α-amino acids with cage carbon frameworks has been performed to date. This paper reports the results of a conformational study on the (R)-8-amino-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-carboxylic acid monopeptide (cage monopeptide), using molecular mechanics and *ab initio* methods. The *in vacuo* Ramachandran maps computed using the different parameterizations of the AMBER force field show the C_{7eq} structure as the most favourable conformation, in contrast to the C_{7ax} structure, that is the lowest energy conformation at the *ab initio* level. Analysis of these maps reveals the helical preference for the monopeptide and provides the potential for the cage residue to be incorporated into constrained peptide analogues.

Keywords: *ab initio* calculations; AMBER force field; cage monopeptide; C$^{\alpha}$-tetrasubstituted α-amino acid; conformational study; unnatural amino acid

INTRODUCTION

The use of unnatural amino acids has expanded the field of peptide design and protein engineering by the addition of new side-chain functionality and a whole variety of conformational profiles. Peptides containing unnatural residues are useful tools for the study of the conformational preferences, the design of bioactive peptide analogues with improved pharmacokinetic profiles or the development of pharmacophore models [1–5]. Moreover, unnatural residues are useful for gaining insight into the role of specific amino acids in protein stability and for engineering new proteins with improved properties [6–8].

The incorporation of cage frameworks into bioactive molecules has been the goal of many research groups over the past few years [9–12]. In the case of peptides, 1-amino-adamantane (compound **1** Figure 1) has been shown to mimic adequately bulky hydrophobic amino acid side chains [13–15]. Moreover, the hydrophobicity of the cage

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skeleton enhances the transport of drugs across cell membranes and increases their affinity for lipophilic regions in receptor molecules [16]. The incorporation of cage frameworks such as the 1-aminoadamantane into drugs should also have the added advantage that metabolic degradation is retarded by the inherent steric bulk of the cage skeleton, thus prolonging the activity and reducing the frequency of drug administration to the patient. On the other hand, incorporation of C^{α}-tetrasubstituted α-amino acids with cage moieties, such as 2-aminoadamantane-2-carboxylic acid (compound **2** Figure 1), into peptides produces receptor site specificity through the induction of a specific conformation to the ligand [17–19]. This has been particularly useful in areas such as antibacterial activity, anabolic action and analgesic activity [16].

As part of a research project aimed at understanding the conformational features induced by the incorporation of conformationally constrained residues into peptides, the conformational profile of different unnatural amino acids was reported recently [21–24]. The present work describes the results of a theoretical conformational study on the C^{α}-tetrasubstituted α-amino acid (R)-8-amino-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-carboxylic acid [20] (compound **3** in Figure 1) assessed by studying the cage monopeptide as a model molecule. To this end the molecule was blocked with an acetyl group on its *N*-terminus and a methylamide group on its *C*-terminus to simulate a peptide extension.

The Ramachandran plot of the monopeptide *in vacuo* was computed using an *ab initio* method at the Hartree-Fock (HF) level using a 6-31G* basis set. In addition, the same map was computed at the molecular mechanics level with two sets of force field parameters (Parm94 and Parm96) with the AMBER program. Maps were compared and used to assess the quality of the force field calculations. Furthermore, these *in vacuo* Ramachandran maps are of limited value for predicting the conformational preferences of amino acids in solution, since the relative stability of the C_5 and C_7 conformers are overestimated in the gas phase due to their characteristic intramolecular hydrogen bond. Therefore, the effect induced by the aqueous solvent on the *ab initio* potential-energy surface was also investigated using the self-consistent reaction field method (SCRF).

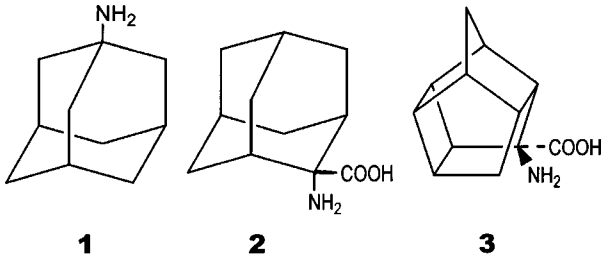

Figure 1 Structures of 1-aminoadamantane (**1**), 2-aminoadamantane-2-carboxylic acid (**2**) and (R)-8-amino-pentacyclo [5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-carboxylic acid (**3**).

METHODS

Ramachandran maps representing the energy as a function of the ϕ and ψ torsion angles of the cage monopeptide were computed at the *ab initio* and molecular mechanics levels. *Ab initio* calculations were performed using the Gaussian 94 suite of programs [25] at the Hartree-Fock level using a 6-31G* basis set. On the other hand, molecular mechanics calculations were carried out with the AMBER 5.0 computer program [26], using both the Parm94 [27] and Parm96 [28] sets of force field parameters.

The structure of the cage monopeptide was first optimized at the *ab initio* level in an extended conformation. This structure was subsequently used to generate the different starting geometries of the Ramachandran map by changing the corresponding dihedral angles systematically on a grid of points on the (ϕ, ψ) space at 30° intervals. At each point of the grid, the geometry was optimized by keeping the dihedral angles, ϕ and ψ, constrained during the minimization process, while allowing all other variables to optimize. Pilot calculations suggested that both standard Parm94 and Parm96 sets of parameters reproduced reasonably well the geometry of the *ab initio* calculations. Accordingly, no extra parameters were introduced. Under these conditions, the Ramachandran map was computed with both sets using a grid of 15°.

After the Ramachandran maps were computed, the geometries of the different minima were identified and characterized by energy optimizations. For the sake of completeness, *ab initio* minimizations were also performed at the Hartree-Fock level using (i) the minimal STO-3G, (ii) the split valence 3-21G and (iii) the polarized 6-31G* basis sets. To investigate the effect of electronic correlation, this property was computed on the optimized HF geometries at the second-order Möller-Plesset (MP2) level of theory.

To perform the molecular mechanics calculations, restrained electrostatic potential (RESP) atomic charges consistent with the Parm94 and Parm96 sets of parameters for the AMBER program were computed by fitting the molecular electrostatic potential (ESP) at the HF level using the 6-31G* basis set. RESP charges were computed using two minimum energy conformations in the fitting procedure [27,28]. To gain insight into the conformational profile of the cage residue, four cross-sections on the potential energy surface were selected. Energy calculations were performed using the AMBER force field, as well as at the HF/6-31G* level.

Solvent effects were computed within the Miertus-Scrocco-Tomasi (MST) SCRF model [29]. This method uses a quantum mechanical description of the solute and a quasi-continuum representation of the solvent. The free energy of solvation is determined from the addition of an electrostatic and a steric term. The MST model may be applied at different levels of quantum mechanics theory. Previous results suggested that the semi-empirical AM1 hamiltonian provides results in good agreement with those obtained at the HF level with a 6-31* basis set for the study of the effect of the aqueous solvent on the potential energy surface of monopeptides [30]. Therefore, the less time consuming MST/AM1 method has been used in the present work.

RESULTS AND DISCUSSION

Figures 2 and 3 show the Ramachandran maps of the residue computed with AMBER using the Parm94 and Parm96 sets of parameters, respectively. Figure 4 shows the map computed at the HF level with a 6-31G* basis set. This basis set was selected for the present calculations since it reproduces all the conformational features of the amino acids at this level of the theory [21]. Accordingly, these calculations were used as the reference in the present work. Energies of the plots were expressed in kcal mol^{-1} relative to the lowest energy minimum in each case, and contours were plotted every 2 kcal mol^{-1}. The letters A–E denote the locations of the different minima on the maps.

A feature revealed by the different calculations is the almost symmetrical shape of the Ramachandran map, due to the nearly symmetrical nature of the

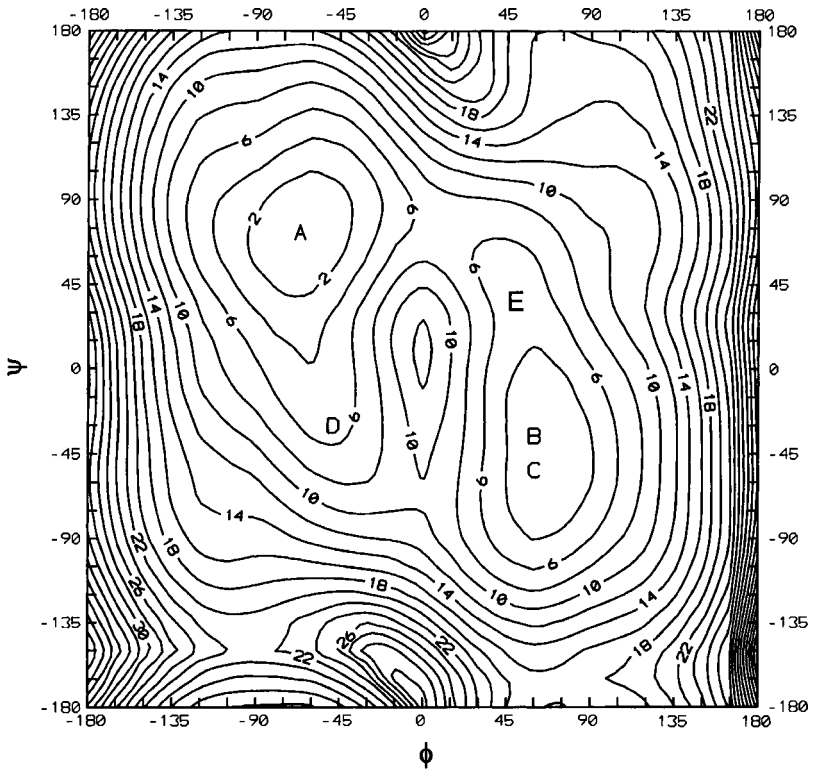

Figure 2 Ramachandran map for Ac-Cage-NHMe computed using the Parm94 force field. Energies relative to the lowest energy minimum are expressed in kcal mol^{-1}. Contours are drawn every 2 kcal mol^{-1}.

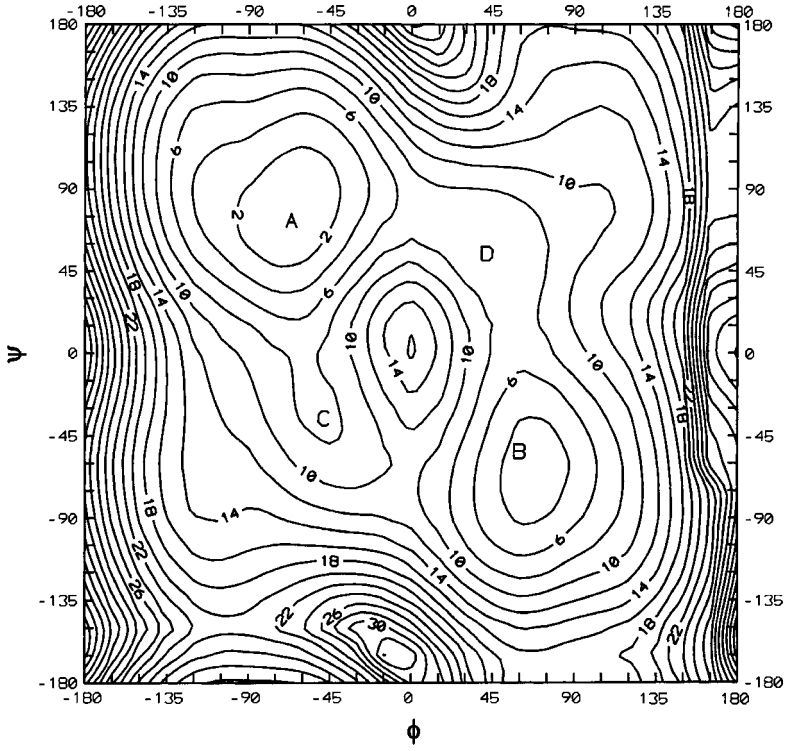

Figure 3 Ramachandran map for Ac-Cage-NHMe computed using the Parm96 force field. Energies relative to the lowest energy minimum are expressed in kcal mol^{-1}. Contours are drawn every 2 kcal mol^{-1}.

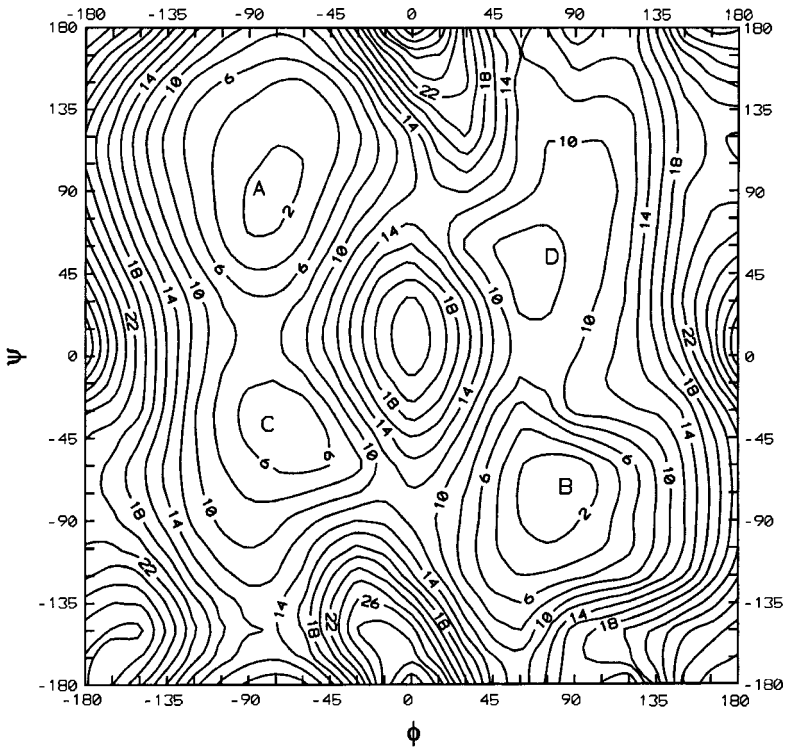

Figure 4 Ramachandran map for Ac-Cage-NHMe computed at the Hartree-Fock level with the 6-31G* basis set. Energies relative to the lowest energy minimum are expressed in kcal mol^{-1}. Contours are drawn every 2 kcal mol^{-1}.

Table 1 Torsional Angles for the Low Energy Conformations of Ac-Cage-NHMe

		Torsional angles (°)							
		C_{7eq}		C_{7ax}		α_R		α_L	
	Method	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
AMBER	Parm94	−61.1	74.9	69.2	−68.8	−50.3	−28.6	44.8	54.3
		—	—	68.8	−40.0	—	—	—	—
	Parm96	−65.3	75.2	65.1	−73.8	−50.0	−30.0	44.9	59.5
Ab initio	HF/STO-3G	−62.3	86.9	62.0	−84.0	−77.9	−50.2	78.8	53.7
	HF/3-21G	−80.4	68.2	78.9	−62.5	−65.3	−37.2	64.1	42.4
	HF/6-31G*	−79.8	79.6	80.2	−69.8	−66.5	−39.9	65.8	43.6

cage residue. Even if there are slight differences in the relative energies of the minima among the different levels of calculation, the symmetry of the map is preserved at the different levels of calculation used.

All the *ab initio* calculations identify the conformation C_7 in the region around $\phi \approx 70°$ and $\psi \approx -70°$ as the global minimum. By analogy with the terminology used for the natural amino acids, this conformation is named as C_{7ax}, although the axial/equatorial designation is not appropriate in the case of C^{α}-tetrasubstituted amino acids. This result can actually be more accurately analysed from the minima listed in Tables 1 and 2. Interestingly, the energy results obtained with the 3-21G basis set at the Hartree-Fock level compare well with the MP2 results obtained using the more extended 6-31G* basis set, suggesting that correlation introduces minimal corrections. At the *ab initio* level structures α_R and α_L are α-helices, whereas at the molecular mechanics level the α_R structure appears rather as a 3_{10}-helix and the α_L structure exhibit low ϕ values. The relative ordering of the low energy conformers predicted at the *ab initio* level is: $C_{7ax} < C_{7eq} < \alpha_R < \alpha_L$, whereas that predicted using the AMBER force fields is: $C_{7eq} < C_{7ax} < \alpha_L < \alpha_R$.

To obtain a deeper understanding into the differences between *ab initio* and molecular mechanics calculations, Figures 5–8 show the energy profiles of cross sections of the Ramachandran map with $\psi = 75°$, $\psi = -45°$, $\phi = 60°$ and $\phi = -90°$ respectively, computed using both the Parm96 force field and at the HF/6-31G* level. The graphs show that, although force field calculations reproduce the location of the *ab initio* minima on the potential energy surface, they are unable to reproduce satisfactorily the relative energies of the different minina. Another differential feature between the two

Table 2 Relative Energies for the Low Energy Conformations of Ac-Cage-NHMe

		Relative energies (kcal mol^{-1})			
	Method	C_{7eq}	C_{7ax}	α_R	α_L
AMBER	Parm94	0.0	2.65	5.17	4.99
	Parm96	0.0	2.86	7.34	5.96
Ab initio	HF/STO-3G	0.52	0.0	2.12	3.14
	HF/3-21G	0.79	0.0	4.75	6.55
	HF/6-31G*	0.86	0.0	3.81	6.32
	MP2/6-31G*	0.93	0.0	4.13	6.68

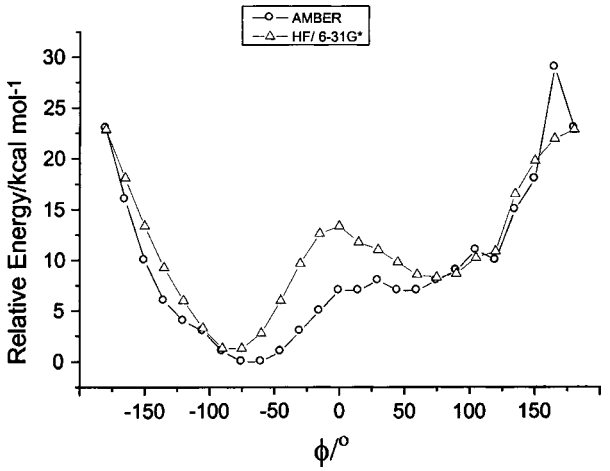

Figure 5 Relative energies on the cross-section of the Ramachandran map for Ac-Cage-NHMe (for $\psi = 75°$), computed at the Hartree-Fock level using the 6-31G* basis set and with AMBER using the Parm96 set of parameters.

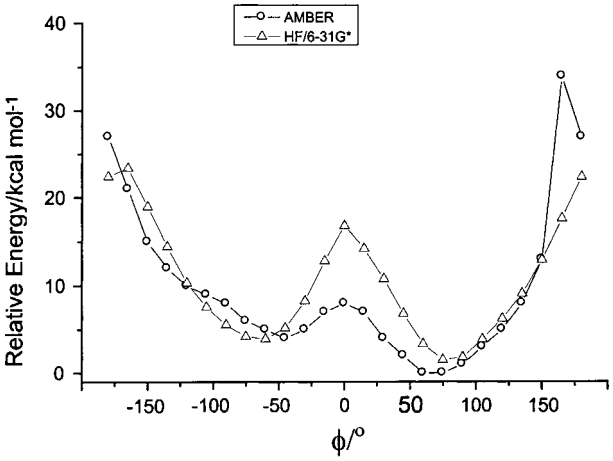

Figure 6 Relative energies on the cross-section of the Ramachandran map for Ac-Cage-NHMe (for $\psi = -45°$) computed at the Hartree-Fock level using the 6-31G* basis set and with AMBER using the Parm96 set of parameters.

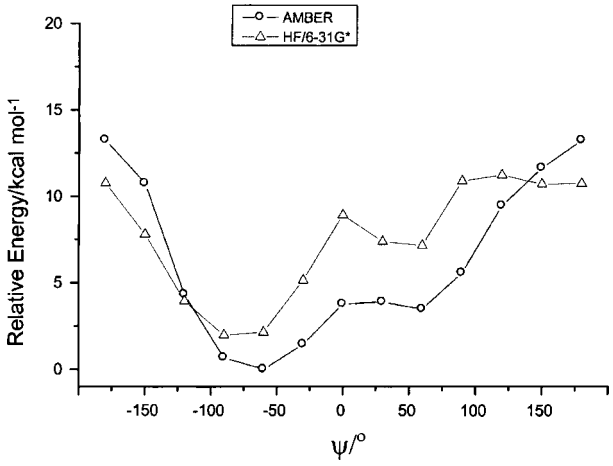

Figure 7 Relative energies on the cross-section of the Ramachandran map for Ac-Cage-NHMe (for $\phi = 60°$) computed at the Hartree-Fock level using the 6-31G* basis set and with AMBER using the Parm96 set of parameters.

kinds of calculations is the height of the energy barriers, much higher in the force field calculations than has been previously reported in similar calculations [21–24].

The most striking feature of the Ramachandran maps shown in Figures 2–4 with regard to standard maps is the absence of the C_5 conformation. This conformation is highly strained in this system due to the repulsive interaction between the amide carbonyl group of the cage residue and a methylene group of the same moiety. The high energy barrier of the ϕ torsion angle in the neighbourhood

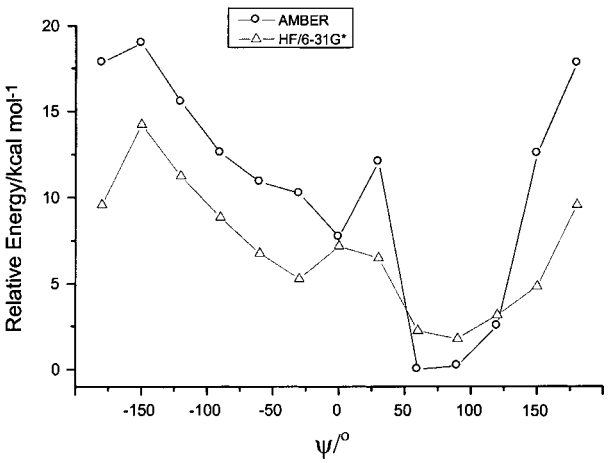

Figure 8 Relative energies on the cross-section of the Ramachandran map for Ac-Cage-NHMe (for $\phi = -90°$) computed at the Hartree-Fock level using the 6-31G* basis set and with AMBER using the Parm96 set of parameters.

of the C_5 conformation is clearly visible in the Ramachandran maps, and reaches its maximum close to 180°.

The four low energy conformations optimized at the HF/6-31G* level are presented in Figures 9–12. Differences in the hydrogen bond characteristics for structures optimized using the Parm94 and Parm96 force fields with respect to those obtained at the HF level using the 6-31G* basis set are presented in Table 3. In general, the intramolecular hydrogen bond parameters at these theoretical levels are in

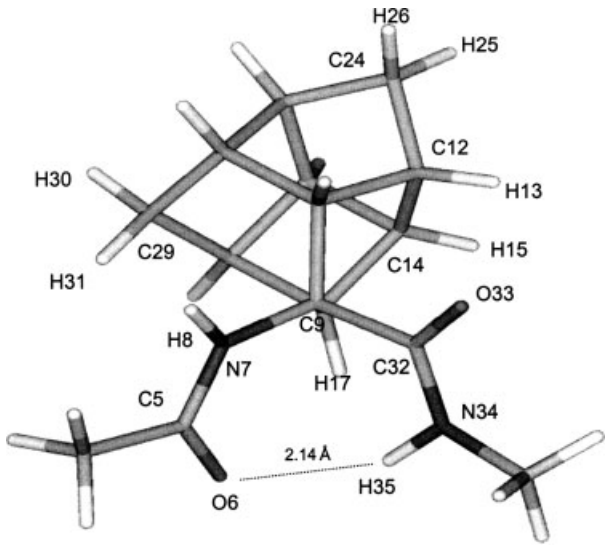

Figure 9 Structure of the C_{7eq} low energy conformation of Ac-Cage-NHMe.

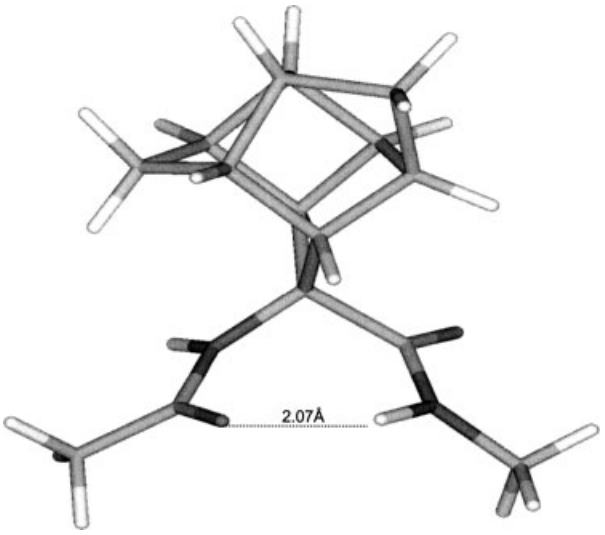

Figure 10 Structure of the C_{7ax} low energy conformation of Ac-Cage-NHMe.

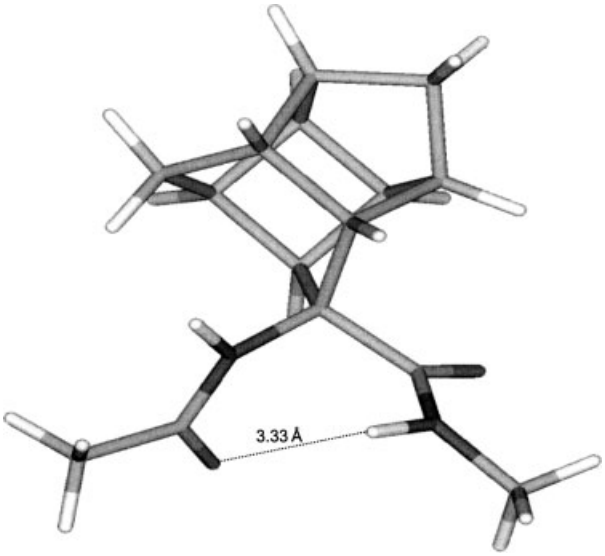

Figure 11 Structure of the α_R low energy conformation of Ac-Cage-NHMe.

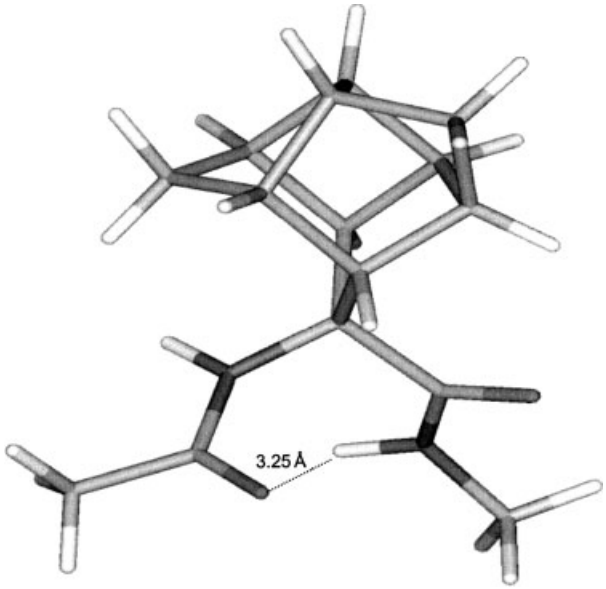

Figure 12 Structure of the α_L low energy conformation of Ac-Cage-NHMe.

good agreement with each other. These values are similar to those obtained for natural amino acids [29]. At the HF/6-31G* level, the low energy C_{7ax} conformation exhibits a hydrogen bond with a H···O distance of 2.07 Å and a < N-H···O angle of 144.3°. The geometry corresponding to a strained C_{7eq} conformation exhibits a hydrogen bond characterized by a H···O distance of 2.14 Å and a <N-H···O angle of 139.9°, being about 0.93 kcal mol^{-1} less stable than the global minimum. Interestingly, the intramolecular hydrogen bond predicted for the C_7 conformation of the Aib monopeptide [29] (H···O distance of 2.01 Å and <N-H···O angle of 148.4°) closely resembles the C_{7ax} conformation obtained at the similar computational level.

The agreement in the ϕ and ψ backbone torsion angles shown in Table 1 for the various theoretical levels is good. Comparison of the geometries of the minima obtained from the different calculations show that there are no significant differences between the results obtained at the molecular mechanics and *ab initio* levels. However, some interesting inferences regarding the bond length variations with conformation can be seen (Table 4). At the HF/6-31G* level, the O6-C5 and N34-H35 bonds for the C_{7ax} and C_{7eq} conformations, which participate in hydrogen bonding, are lengthened compared with the C32-O33 and N7-H8 bonds, which are not hydrogen bonded. This effect is to be expected from the charge polarization effect, with a similar result obtained for N^{α}-acetyl-N'-methylalanineamide [31]. Also at the same level, the peptide bond lengths C5-N7 and C32-N34 are shorter for the low energy structures (C_{7ax} and C_{7eq}) and longer for the high energy structures (α_R and α_L). Examination of the helical conformers (Figures 11 and 12) show that the amide hydrogen H35 lies directly in the region of space occupied by the lone pair orbital associated with the amide nitrogen N7. Interestingly, the H35···N7 distance of 2.33 Å is similar to the corresponding distance measured

Table 3 Hydrogen Bond Characteristics for the Low Energy Conformations of Ac-Cage-NHMe

	Method	C_{7eq}	C_{7ax}	α_R	α_L
$d(H_{35}\ldots O_6)$ (Å)	Parm94	2.02	1.94	2.71	3.39
$(N_{34}-H_{35}\ldots O_6)$ (°)		131.7	142.8	96.3	73.65
$d(H_{35}\ldots O_6)$ (Å)	Parm96	2.04	1.97	2.76	3.54
$(N_{34}-H_{35}\ldots O_6$ (°)		133.0	137.2	94.4	70.8
$d(H_{35}\ldots O_6)$ (Å)	HF/6-31G*	2.14	2.07	3.33	3.25
$(N_{34}-H_{35}\ldots O_6)$ (°)		139.9	144.3	87.6	89.6

Table 4 Calculated Bond Lengths for the Low Energy Conformations of Ac-Cage-NHMe

	C_{7eq}		C_{7ax}		α_R		α_L	
	AMBER	HF/6-31G*	AMBER	HF/6-31G*	AMBER	HF/6-31G*	AMBER	HF/6-31G*
C5-C2	1.509	1.514	1.510	1.514	1.511	1.515	1.510	1.515
O6-C5	1.223	1.207	1.223	1.207	1.220	1.198	1.221	1.198
N7-C5	1.334	1.348	1.333	1.347	1.333	1.360	1.333	1.359
H8-N7	1.007	0.994	1.006	0.994	1.007	0.993	1.006	0.993
C9-N7	1.473	1.465	1.473	1.464	1.479	1.458	1.476	1.460
C10-C9	1.56	1.542	1.557	1.549	1.557	1.549	1.561	1.550
C32-C9	1.557	1.550	1.555	1.549	1.565	1.547	1.559	1.552
O33-C32	1.23	1.204	1.229	1.204	1.230	1.200	1.230	1.198
N34-C32	1.337	1.347	1.339	1.345	1.341	1.353	1.340	1.356
H35-N34	1.016	0.997	1.017	0.997	1.007	0.992	1.006	0.992
C36-N34	1.46	1.447	1.461	1.446	1.461	1.447	1.461	1.447

for N^{α}-acetyl-N'-methylalanine amide by Scarsdale *et al.* [31].

The largest variations of the ϕ and ψ values between the AMBER and the STO-3G results are about 20°, while those between AMBER and the HF/6-31G* level are about 15°. The most distinct difference for the results shown in Table 2 is that the molecular mechanics calculations are able to predict the C_{7eq} conformer as being favoured by about 2.7 kcal mol^{-1} with respect to the C_{7ax} conformer, whereas at the *ab initio* level the C_{7ax} is favoured by less than 1 kcal mol^{-1} with respect to the C_{7eq} conformer. The results also show that the α_L conformer is favoured by about 1 kcal mol^{-1} over the α_R conformer at the molecular mechanics level, while at the *ab initio* level the α_R conformer is about 2 kcal mol^{-1} more stable than the α_L conformer. The stability of the C_7 conformation over the helical conformation is evident. This result is expected as it is well known that the hydrogen-bonded conformations *in vacuo* are energetically more favoured.

There is a differential feature between the two AMBER calculations. The Ramachandran map computed using the Parm94 set of parameters exhibits five unique minima (C_{7eq}, α_R, α_L and two in the C_{7ax} region), while only four minima are characterized using the Parm96 set of parameters. This difference is not very significant, since the two minima characterized with the Parm94 set in the C_{7ax} region are degenerate over a wide range of ψ values.

Figure 13 shows the *ab initio* 6-31G* Ramachandran map corrected with the MST/AM1 free energy of solvation in water. As expected, the aqueous solvation introduces a relative stabilization of the helical structures, characterized by the alignment in a parallel orientation and the exposition to the polar solvent of the dipolar amide groups. On the contrary, the C_7 structures, that are favoured in the gas-phase map due to the formation of the intramolecular hydrogen bond, are less stabilized in the aqueous solvent. In conclusion, the solvent lowers the right-

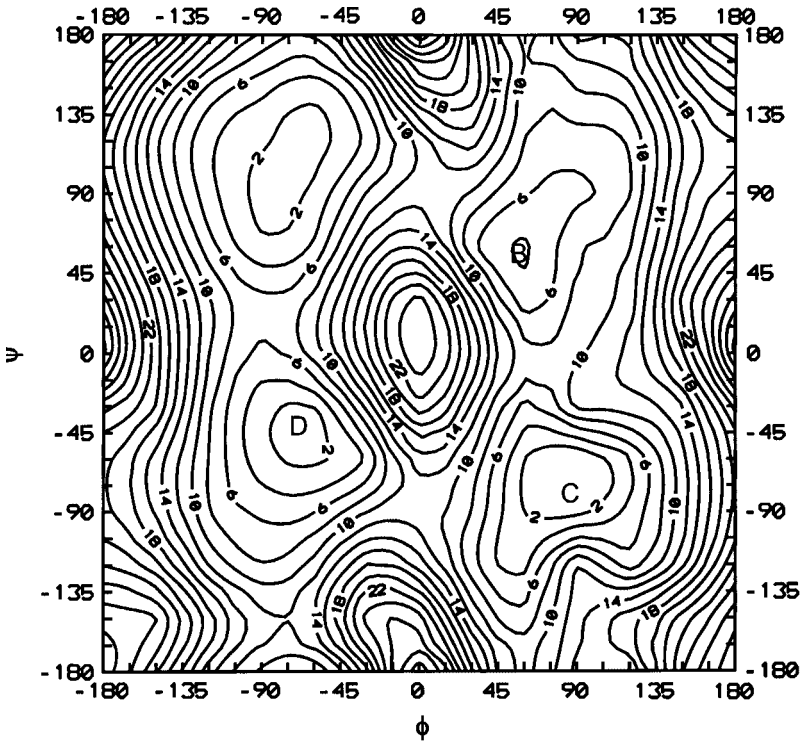

Figure 13 Ramachandran map for Ac-Cage-NHMe computed as the sum of the *ab initio* HF/6-31G* map and the AM1/MST free energy of solvation. Contours are drawn every 2 kcal mol^{-1}.

and left-handed helix minima. Similar effects have been described for glycine and alanine monopeptides in a reaction field model of aqueous solvent [32]. The region corresponding to the right-hand helix is only about 1.5 kcal/mol above the region of the C_{7ax} conformation and more than 20 kcal/mol below the energies corresponding to the C_5 structure (not a minimum). These results suggest that the cage amino acid would preferentially induce helical structures in peptides in aqueous solution.

CONCLUSIONS

The performance of the molecular mechanics method using the standard force field parameters to describe the cage residue was assessed by comparison with *ab initio* calculations. AMBER-computed conformations of the cage residue are comparable to those obtained at the *ab initio* level. However, the AMBER force field favours the C_{7eq} structure in comparison with the C_{7ax} structure. This result is in contrast to what is observed at the *ab initio* level, the difference in relative energy being about 1 kcal mol^{-1}. The cage residue has the tendency to induce an α_R-helix, as well as an α_L-helix, to the peptide chain. Furthermore, the results clearly show that the cage residue does not favour the C_5 (fully extended) conformation.

The introduction of a bulky cage in the amino acid structure causes strong effects on conformational preferences. The main consequence is the destabilization of the extended conformations with ϕ values near 180°. Therefore, the cage amino acid inhibits the formation of β-sheet structures in peptides. In fact, its conformational space is very restricted: the *ab initio* map shows that only the C_7 and the right-handed helical regions have energies less that 6 kcal/mol above the absolute minimum. The high stability of the C_7 conformations, found in all calculations of amino acids, is a drawback of the *in vacuo* calculations. Taking into account that these conformations usually do not contribute to the secondary structure of peptides, it can be predicted that the cage amino acid will preferentially induce helical structures.

The conformational map computed with the AMBER force field reproduces reasonably well the general patterns of the *ab initio* results. However, the relative order of the minima and their relative

energies are different, suggesting that it would be necessary to develop specific parameters to model this kind of amino acid.

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